IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Mitani et al.	Examiner:	Bertagna, Angela
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Title:	PROCESS FOR AMPLIFYING NUCLEIC ACIDS		

DECLARATION UNDER 37 CFR §1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

I, Takefumi Ishidao, hereby declare as follows:

1. I graduated from
1996, March; Department of Biological Science, Faculty of Science, Kumamoto University.
1998, March; Master's Course, Graduate School of Science and Technology, Kumamoto University.
2001, March; Doctor's Course, Department of Biological Sciences, Graduate School of Science, and Faculty of Science Osaka University.

2. I have worked in
2001, April; Department of Molecular and Genetic Information, Institute of Molecular and Cellular Biosciences, Tokyo University
2004, April; Laboratory of Molecular Genetics, RIKEN Tsukuba Institute
2007, September; Kabushiki Kaisya DNAFORM.

3. Under my direction, the following experiments were conducted for the purposes of demonstrating the effects of claims 1 and 9 of US Application No. 10/532975.

Experiment I: amplification studies of the sY160 gene using Human DNA as a template was conducted (experiments related to data shown in Table 3 of Technical Explanation that was discussed during interview on January 21, 2010).

Experiment II: amplification studies of sY153 of the Human STS DYS 237 gene using Human DNA as a template was conducted (experiments related to data shown in Table 4 of Technical Explanation that was discussed during interview on January 21, 2010).

Experiment I

1. Experiment Objective

To demonstrate the effects of the invention according to the claims in US Application No. 10/532975 by amplifying the sY160 gene using Human DNA (manufactured by Clontech) as a template.

2. Experimental Method

In this example, it was attempted to amplify the sY160 gene using Human DNA (manufactured by Clontech) as a template. The primer used was as described below. We requested Operon Biotechnologies to synthesize these primers.

The features of the primers used for the experiments are described below. Furthermore, the relationships of respective primers to the template were as illustrated in FIG. 1. In this connection, underlined parts in the following sequences represent 3'-end regions common to each of sense primers and antisense primers, respectively.

Primer Set 1: a combination of a sense primer in which after annealing of a sequence (20mer) placed in the 3'-end side of a primer to the template and extension reaction, a sequence (13mer) in the 5'-end side is hybridized with a region starting from 27 bases downstream of the 3'-end portion of the primer on a strand extended from the primer, and an antisense primer in which after annealing of a sequence (20mer) placed in the 3'-end side of a primer to the template and extension reaction, a sequence (13mer) in the 5'-end side is hybridized with a region starting from 21 bases downstream of the 3'-end portion of the primer on a strand extended from the primer:

SY160LP13 : ATTCGATTCCGTTTACGGGTCTCGAATGGAATA

SY160RP13 : CTAAATCGAATGGTCATTGCATTCCTTTCCATT

Primer Set 2: a combination of a sense primer in which after annealing of a sequence (20mer) placed in the 3'-end side of a primer to the template and extension reaction, a sequence (16mer) in the 5'-end side is hybridized with a region starting from 27 bases downstream of the 3'-end portion of the primer on a strand extended from the primer, and an antisense primer in which after annealing of a sequence (20mer) placed in the 3'-end side of a primer to the template and extension reaction, a sequence (16mer) in the 5'-end side is hybridized with a region starting from 21 bases downstream of the 3'-end portion of the primer on a strand extended from the primer:

SY160LP16 : GACATTCGATTCCGTTTACGGGTCTCGAATGGAATA

SY160RP16 : GAACTAAATCGAATGGTCATTGCATTCCTTTCCATT

Primer Set 3: a combination of a sense primer in which after annealing of a sequence (20mer) placed in the 3'-end side of a primer to the template and extension reaction, a sequence (13mer) in the 5'-end side is hybridized with a region starting from 1 base downstream of the 3'-end portion of the primer on a strand extended from the primer, and an antisense primer in which after annealing of a sequence (20mer) placed in the 3'-end side of a primer to the template and extension reaction, a sequence (13mer) in the 5'-end side is hybridized with a region starting from 1 base downstream of the 3'-end portion of the primer on a strand extended from the primer:

SY160LPY013 : TTCCATATATTTTTACGGGTCTCGAATGGAATA

SY160RPY013 : TTGATAGGAACGGTCATTGCATTCCTTTCCATT

Primer Set 4: a combination of a sense primer in which after annealing of a sequence (20mer) placed in the 3'-end side of a primer to the template and extension reaction, a sequence (16mer) in the 5'-end side is hybridized with a region starting from 1 base downstream of the 3'-end portion of the primer on a strand extended from the primer, and an antisense primer in which after annealing of a sequence (20mer) placed in the 3'-end side of a primer to the template and extension reaction, a sequence (16mer) in the 5'-end side is hybridized with a region starting from 1 base downstream of the 3'-end portion of the primer on a strand extended from the primer:

SY160LPY016 : CCATTCCATATATTTTTACGGGTCTCGAATGGAATA

SY160RPY016 : GAATTGATAGGAACGGTCATTGCATTCCTTTCCATT

Table 1 summarizes the features of the above-mentioned primer sets 1 to 4. In Table 1, "o" denotes that the primer satisfies the following mathematical formula 1 or 2 as recited in claims 1 and 9 of US Application No. 10/532975 (hereinafter referred to as a "present patent

application"). Furthermore, "×" denotes that the primer does not satisfy the following mathematical formula 1 or 2. In this case, as can be seen from Table 1, all the primers satisfy the condition of $10 \leqq X \leqq 30$.

Mathematical Formula 1: $-1.00 \leqq (X-Y)/X \leqq 0.75$
Mathematical Formula 2: $30 \leqq X+Y \leqq 50$

Table 1

Primer set	Primer	X	Y	(X-Y)/X	X+Y	Formula 1	Formula 2
1	SY160LP13	20	26	−0.3	46	○	○
	SY160RP13	20	20	0	40		
2	SY160LP16	20	26	−0.3	46	○	○
	SY160RP16	20	20	0	40		
3	SY160LPY013	20	0	1	20	×	×
	SY160RPY013	20	0	1	20		
4	SY160LPY016	20	0	1	20	×	×
	SY160RPY016	20	0	1	20		

<Amplification Experiment>

A reaction solution (25 μL) with the following composition was prepared: Tris-HCl (20 mM, pH 8.8), KCl (10 mM), $(NH_4)_2SO_4$ (10 mM), $MgSO_4$ (2 mM), Triton X-100 (0.1%), dNTP (0.4 mM), 100 pmol of each of the above-mentioned primer pairs, 100 ng of template DNA, and 8 U of Bst DNA polymerase (NEW ENGLAND BioLabs). This was incubated at 60°C for 60 minutes, 90 minutes, or 120 minutes.

Then 5 μl of each reaction solution was electrophoresed with 3% NuSieve GTG Agarose (manufactured by BMA; purchased from TAKARA BIO INC.; "NuSieve" is a registered trademark of BMA).

<Amplification Product Verification Test>

Subsequently to the above-mentioned amplification experiment, a further amplification product verification test was conducted as follows. That is, among the amplification products obtained in the above-mentioned amplification experiment, the amplification product that seemed to have the highest amplification efficiency as an amplification experiment object in Table 1 was used and digested with a restriction enzyme. Conditions for digestion with the

restriction enzyme were 37°C for 3 hours, and 1 μL of reaction solution of the amplification product obtained using each of the primer sets was digested with a restriction enzyme BstXI.

Each digested product was electrophoresed with 3% NuSieve GTG Agarose (manufactured by BMA; purchased from TAKARA BIO INC.; "NuSieve" is a registered trademark of BMA).

3.Experimental Result

<Description of Figures>

FIG. 1 below shows the relationships of respective primers to the template in the primer sets 1 to 4.

FIG. 2 below is an agarose gel electrophoresis showing the results of the amplification experiments with the primer sets 1 to 4. In FIG. 2, the numerical values indicated on the left side of the electrophoresis denote the sizes of the DNA size marker. Furthermore, the table shown below the gel indicates reaction conditions of each primer in the amplification experiment. In the table, with respect to the template, "y" indicates that the template was added as described above, while "n" indicates that the same reaction was performed with no template added.

FIG. 3 below is an agarose gel electrophoresis showing the results with respect to the primer set 2. The sample of each lane shown in FIG. 3 is as in the legend below FIG. 3. The numerical values indicated on the right side of the gel are the speculated sizes of the restriction digestion fragments and confirms that the targeted amplified product was obtained.

<Amplification Experiment Results>

In the reaction with no template being added, no band other than that in which an unreacted primer was dyed was observed. The results thereof are shown in lanes 5, 9, 13, and 17 (primer sets 1 to 4) in FIG. 2.

In each of the primer sets 1 and 2, which is composed of primers that satisfy both the mathematical formulae 1 and 2, a target amplification product was obtained sufficiently through a reaction in a short reaction time of 90 minutes after a template was added. The results thereof are shown in lanes 3 and 4 (primer set 1) and lanes 7 and 8 (primer set 2) in FIG. 2. Among small size bands, the band around 260 bp indicates a product anticipated by the synthesis reaction of the present invention.

In the primer sets 3 and 4 (both having Y=0) composed of primers that satisfy neither the mathematical formula 1 nor the mathematical formula 2, an amplification product was obtained only in the sample subjected to a reaction time of 120 minutes. These results are shown in lane 12 (primer set 3) and lane 16 (primer set 4) in FIG. 2.

<Amplification Product Verification Test Results>

In the primer set 2, which satisfies both the mathematical formulae 1 and 2, most of the bands in the undigested state were changed into those with sizes estimated to be obtained after digestion with the restriction enzyme. The results thereof are shown in lane 2 (primer set 2) in FIG. 3. Thus, it was proved that target amplification products were obtained efficiently through reactions in a short reaction time of 90 minutes using this primer set.

As described above, with the primer sets that satisfy both the mathematical formulae 1 and 2, a target amplification product was obtained sufficiently in a reaction time as short as 90 minutes. On the other hand, with primer sets that include primers that have Y=0 and do not satisfy the mathematical formulas 1 and 2, the reaction time was 120 minutes and the amplification efficiency was inferior. Thus, according to the inventions of the claims of the present patent application, it was confirmed that the desired effects (performances) were obtained in the amplification of the sY160 gene using Human DNA as a template.

Figure 1

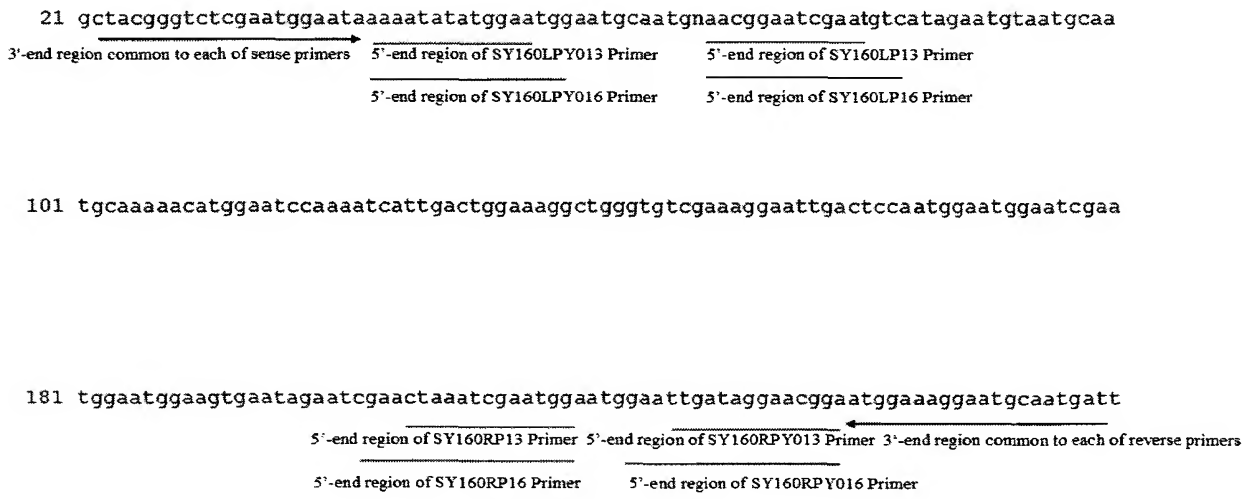
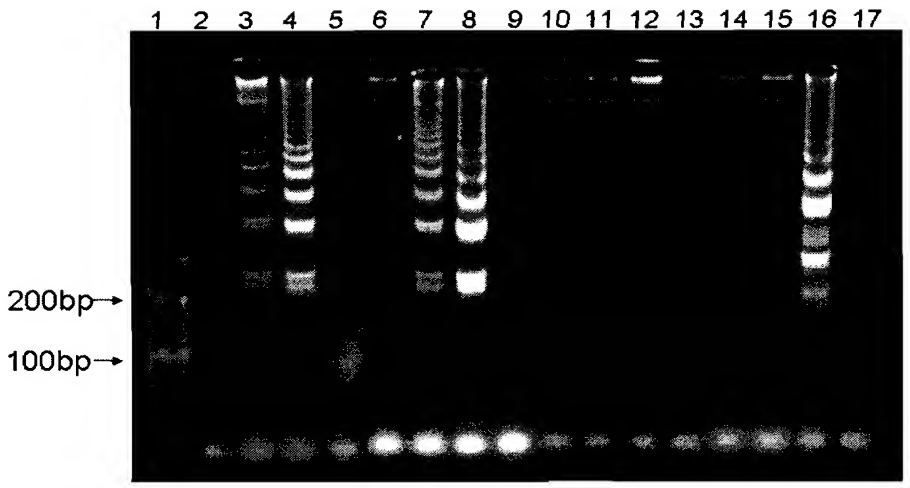
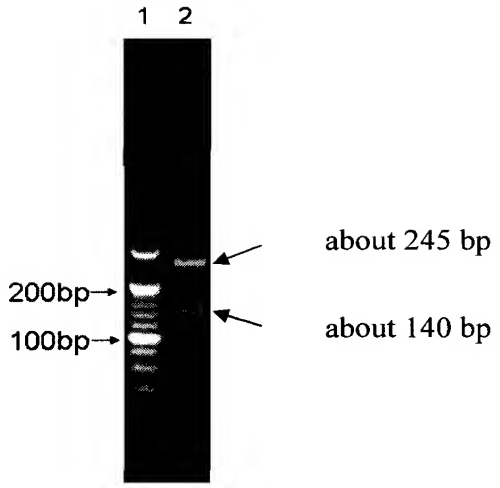


Figure 2



Lane	Primer Sets	Template Present(Y/N)	Reaction Time (Minutes)
1	DNA size marker		
2	Primer Set 1	y	60
3	Primer Set 1	y	90
4	Primer Set 1	y	120
5	Primer Set 1	n	120
6	Primer Set 2	y	60
7	Primer Set 2	y	90
8	Primer Set 2	y	120
9	Primer Set 2	n	120
10	Primer Set 3	y	60
11	Primer Set 3	y	90
12	Primer Set 3	y	120
13	Primer Set 3	n	120
14	Primer Set 4	y	60
15	Primer Set 4	y	90
16	Primer Set 4	y	120
17	Primer Set 4	n	120

Figure 3



Legend for Figure 3
Lane 1: DNA size marker
Lane 2: amplified products of sY160 treated with a restriction enzyme

Experiment II

1. Experiment Objective

To demonstrate the effects of the invention according to claims 1 and 9 in US Application No. 10/532975 by amplifying sY153 of the human STS DYS237 gene using Human DNA (manufactured by Clontech) as a template.

2. Experimental Method

In this example, it was attempted to amplify the sY153 using Human DNA (manufactured by Clontech) as a template. The primer used was as described below. We requested Operon Biotechnologies to synthesize these primers.

The features of the primers used for the experiments are described below. Furthermore, the relationships of respective primers to the template were as illustrated in FIG. 1 below (same as Figure 2 of present patent application). The primers were designed so that an intervening sequence AA was inserted between the sequence on the 3' end side (sequence that hybridizes to

the sequence on the 3' end portion of the targeted nucleic acid sequence) and the sequence on the 5' end side (sequence that hybridizes to the primer elongated strand) on each of the primers, and used in the experiments as described below. In addition, as to the sequences below, underlined parts in the following sequences represent 3'-end regions common to each of sense primers and antisense primers, respectively, and the intervening sequence AA is indicated in bold italics.

Primer Set 1: a combination of primers in which after annealing of a sequence (20mer) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (13mer) on the 5'-end side is hybridized with a region starting from 1 base downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP13-0A2 AAGTCTCTGATGT***AA***GCATCCTCATTTTATGTCCA

SY153RP13-0A2 AGAACTCGCTTTA***AA***CAACCCAAAAGCACTGAGTA

Primer Set 2: a combination of primers in which after annealing of a sequence (20mer: same sequence as Primer Set 1) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (13mer) on the 5'-end side is hybridized with a region starting from 6 bases downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP13-5A2 GTATTAAGTCTCT***AA***GCATCCTCATTTTATGTCCA

SY153RP13-5A2 CACTAAGAACTCG***AA***CAACCCAAAAGCACTGAGTA

Primer Set 3: a combination of primers in which after annealing of a sequence (20mer: same sequence as Primer Set 1) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (13mer) on the 5'-end side is hybridized with a region starting from 11 bases downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP13-10A2 GTTCAGTATTAAG***AA***GCATCCTCATTTTATGTCCA

SY153RP13-10A2 AGCATCACTAAGA***AA***CAACCCAAAAGCACTGAGTA

Primer Set 4: a combination of primers in which after annealing of a sequence (20mer: same sequence as Primer Set 1) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (13mer) on the 5'-end side is hybridized with a region starting

from 16 bases downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP13-15A2	CATTTGTTCAGTA*AA*GCATCCTCATTTTATGTCCA
SY153RP13-15A2	CTTGCAGCATCAC*AA*CAACCCAAAAGCACTGAGTA

Primer Set 5: a combination of primers in which after annealing of a sequence (20mer: same sequence as Primer Set 1) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (10mer) on the 5'-end side is hybridized with a region starting from 21 bases downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP10A2	GGCATTTGTT*AA*GCATCCTCATTTTATGTCCA
SY153RP10A2	ATCTTGCAGC*AA*CAACCCAAAAGCACTGAGTA

Primer Set 6: a combination of primers in which after annealing of a sequence (20mer: same sequence as Primer Set 1) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (13mer) on the 5'-end side is hybridized with a region starting from 21 bases downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP13A2	TGTGGCATTTGTT*AA*GCATCCTCATTTTATGTCCA
SY153RP13A2	AACATCTTGCAGC*AA*CAACCCAAAAGCACTGAGTA

Primer Set 7: a combination of primers in which after annealing of a sequence (20mer: same sequence as Primer Set 1) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (16mer) on the 5'-end side is hybridized with a region starting from 21 bases downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP16A2	TTATGTGGCATTTGTT*AA*GCATCCTCATTTTATGTCCA
SY153RP16A2	CTTAACATCTTGCAGC*AA*CAACCCAAAAGCACTGAGTA

Primer Set 8: a combination of primers in which after annealing of a sequence (20mer: same sequence as Primer Set 1) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (22mer) on the 5'-end side is hybridized with a region starting

from 21 bases downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP22A2 TTACCTTTATGTGGCATTTGTT*AA*GCATCCTCATTTTATGTCCA

SY153RP22A2 ATTTAACTTAACATCTTGCAGC*AA*CAACCCAAAAGCACTGAGTA

Primer Set 9: a combination of primers in which after annealing of a sequence (20mer: same sequence as Primer Set 1) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (25mer) on the 5'-end side is hybridized with a region starting from 21 bases downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP25A2

TCATTACCTTTATGTGGCATTTGTT*AA*GCATCCTCATTTTATGTCCA

SY153RP25A2

AAGATTTAACTTAACATCTTGCAGC*AA*CAACCCAAAAGCACTGAGTA

Primer Set 10: a combination of primers in which after annealing of a sequence (20mer: same sequence as Primer Set 1) placed on the 3'-end side of each of the primers to the template and extension reaction, a sequence (28mer) on the 5'-end side is hybridized with a region starting from 21 bases downstream of the 3'-end portion of each of the primers on a strand extended from the respective primers, and an intervening sequence AA is inserted in between the sequence on the 3' end side and the sequence of the 5'end side of these primers:

SY153LP28A2

CAGTCATTACCTTTATGTGGCATTTGTT*AA*GCATCCTCATTTTATGTCCA

SY153RP28A2

AAGAAGATTTAACTTAACATCTTGCAGC*AA*CAACCCAAAAGCACTGAGTA

Table 1 is shown below in which features of the above-mentioned primer sets 1 to 10 are summarized. In the following tables 1, "○" denotes that the primer satisfies the following mathematical formula 1 or 2 described in the claims of the present patent application. Furthermore, "×" denotes that the primer does not satisfy the following mathematical formula 1 or 2. In this case, as can be seen from the following Table 1, all the primers satisfy the condition of $10 \leqq X \leqq 30$.

Formula 1: $-1.00 \leqq \{X-(Y-Y')\}/X \leqq 0.75$
Formula 2: $30 \leqq X+Y+Y' \leqq 50$

Table 1

Primer Sets	Primers	X	Y	Inter-vening Seq Y'	{X-(Y-Y')}/X	X+Y+Y'	Formula 1	Formula 2
1	sY153Lp13-5A2	20	0	2	1.1	22	×	×
	sY153Rp13-0A2	20	0	2	1.1	22		
2	sY153Lp13-5A2	20	5	2	0.85	27	×	×
	sY153Rp13-5A2	20	5	2	0.85	27		
3	sY153Lp13-10A2	20	10	2	0.6	32	○	○
	sY153Rp13-10A2	20	10	2	0.6	32		
4	sY153Lp13-15A2	20	15	2	0.35	37	○	○
	sY153Rp13-15A2	20	15	2	0.35	37		
5	sY153Lp10A2	20	20	2	0.1	42	○	○
	sY153Rp10A2	20	20	2	0.1	42		
6	sY153Lp13A2	20	20	2	0.1	42	○	○
	sY153Rp13A2	20	20	2	0.1	42		
7	sY153Lp16A2	20	20	2	0.1	42	○	○
	sY153Rp16A2	20	20	2	0.1	42		
8	sY153Lp22A2	20	20	2	0.1	42	○	○
	sY153Rp22A2	20	20	2	0.1	42		
9	sY153Lp25A2	20	20	2	0.1	42	○	○
	sY153Rp25A2	20	20	2	0.1	42		
10	sY153Lp28A2	20	20	2	0.1	42	○	○
	sY153Rp28A2	20	20	2	0.1	42		

<Amplification Experiment>

A reaction solution (25 μL) with the following composition was prepared: Tris-HCl (20 mM, pH 8.8), KCl (10 mM), $(NH_4)_2SO_4$ (10 mM), $MgSO_4$ (2 mM), Triton X-100 (0.1%), dNTP (0.4 mM), 100 pmol of each of the above-mentioned primer pairs, 100 ng of template DNA, and 8 U of Bst DNA polymerase (NEW ENGLAND BioLabs). This was incubated at 60°C for 20 minutes, 40 minutes, or 60 minutes.

Then 5 μl of each reaction solution was electrophoresed with 3% NuSieve GTG Agarose (manufactured by BMA; purchased from TAKARA BIO INC.; "NuSieve" is a registered trademark of BMA).

<Amplification Product Verification Test>

Subsequently to the above-mentioned amplification experiment, further an amplification product verification test was conducted as follows. That is, among the amplification products obtained in the above-mentioned amplification experiment, the amplification product that seemed to have highest amplification efficiency was used and digested with a restriction enzyme. Conditions for digestion with the restriction enzyme were 37°C for 3 hours, and 1 μL of reaction solution of the amplification product obtained using each of the primer sets was digested with a restriction enzyme BstXI.

Each digested product was electrophoresed with 3% NuSieve GTG Agarose (manufactured by BMA; purchased from TAKARA BIO INC.; "NuSieve" is a registered trademark of BMA).

3. Experimental Result

<Description of Figures>

FIGS. 2 and 3 below are agarose gel electrophoresis showing the results of the amplification experiments with the primer sets 1 to 10. The gel in FIG. 2 shows the results obtained with the primer sets 1 to 5, and the gel in FIG. 3 shows the results obtained with the primer sets 6 to 10. In each figure, the numerical values indicated on the left side of the gel denote the sizes of the DNA size marker. Furthermore, in each figure, the table shown below the electrophoregram indicates reaction conditions of each primer in the amplification experiment. In the tables, with respect to the template, "y" indicates that the template was added as described above, while "n" indicates that the same reaction was performed with no template added.

FIG. 4 below is an agarose gel electrophoresis showing the result with respect to the primer set 4. The sample of each lane shown in FIG. 4 is as in the legend below FIG. 4. The numerical values indicated on the right side of the gel are the speculated sizes of the restriction digestion fragments and confirms that the targeted amplified product was obtained.

<Amplification Experiment Results>

In the reaction with no template being added, no band other than that in which an unreacted primer was dyed was observed. The results thereof are shown in lanes 5, 9, 13, 17,and 21 (primer sets 1 to 5) in FIG. 2 and lanes 5, 9, 13, 17 and 21 (primer sets 6 to 10) in FIG. 3.

In each of the primer sets 3 to 10, which is composed of primers that satisfy both the mathematical formulae 1 and 2, a target amplification product was obtained sufficiently through a reaction in a short reaction time of 40 minutes after a template was added. The results thereof are shown in lanes 11, 12, 15, 16, 19 and 20 (primer sets 3-5) in FIG. 2 and lanes 3, 4, 7, 8, 11, 12, 15, 16, 19 and 20 (primer sets 6-10) in FIG. 3. Among small size bands, the band around 160 bp indicates a product anticipated by the synthesis reaction of the present invention.

12, 15, 16, 19 and 20 (primer sets 6-10) in FIG. 3. Among small size bands, the band around 160 bp indicates a product anticipated by the synthesis reaction of the present invention.

In the primer set 2 composed of primers that satisfy neither the mathematical formula 1 nor the mathematical formula 2, an amplification product was obtained only in the sample subjected to a reaction time of 60 minutes (lane 8 in Figure 2), and in primer set 1, no amplification product was obtained in the sample subjected to a reaction time of 60 minutes (lane 4 in Figure 2).

<Amplification Product Verification Test Results>

In the primer set 4, which satisfies both the mathematical formulae 1 and 2, most of the bands in the undigested state were changed into those with sizes estimated to be obtained after digestion with the restriction enzyme. The results thereof are shown in lane 2 (primer set 4) in FIG. 4. Thus, it was proved that target amplification products were obtained efficiently through reactions in a short reaction time of 40 minutes using this primer set.

As described above, with the primer sets that satisfy both the mathematical formulae 1 and 2 and include an intervening sequence, a target amplification product was obtained sufficiently in a reaction time as short as 40 minutes. On the other hand, with primer sets that include an intervening sequence and do not satisfy the mathematical formulas 1 and 2, the reaction time was 60 minutes or amplification was not obtained, and the amplification efficiency was inferior. Thus, according to the inventions of the claims of the present patent application, it was confirmed that the desired effects (performances) were obtained in the amplification of the sY153 using Human DNA as a template, even with primers that include an intervening sequence.

Figure 1

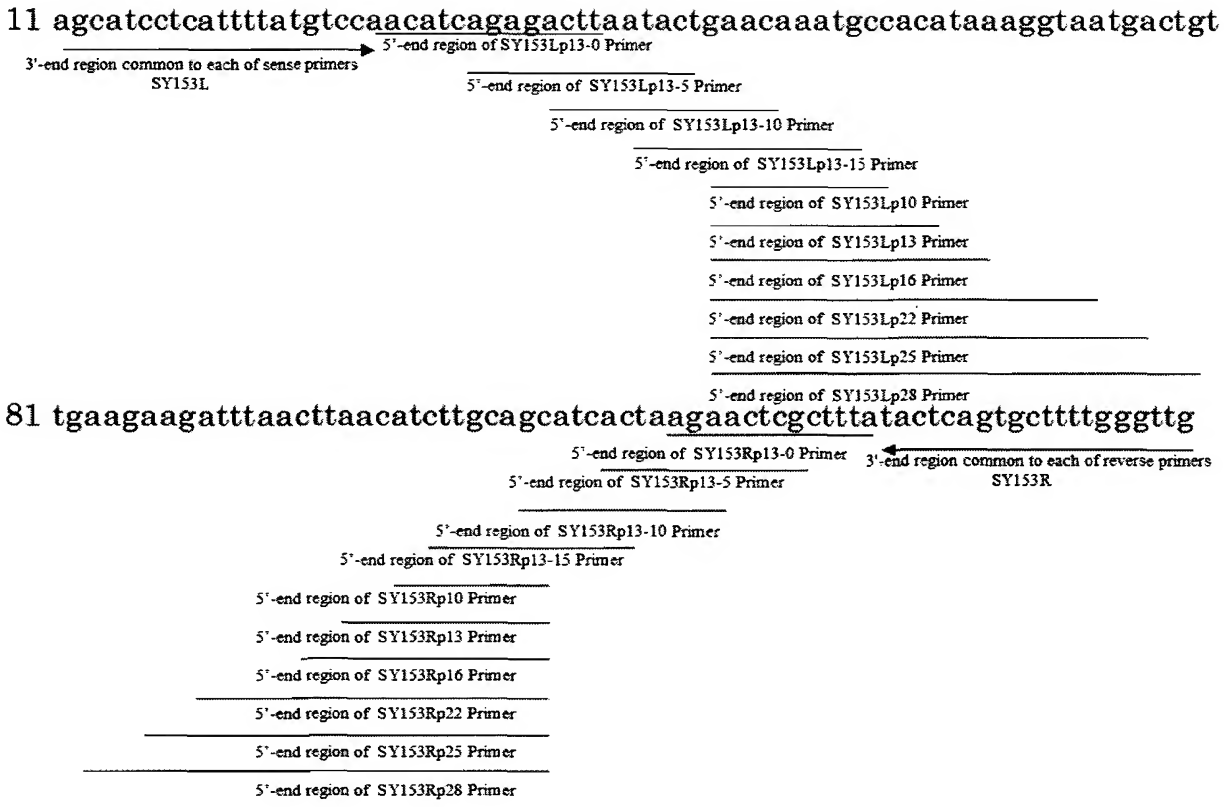
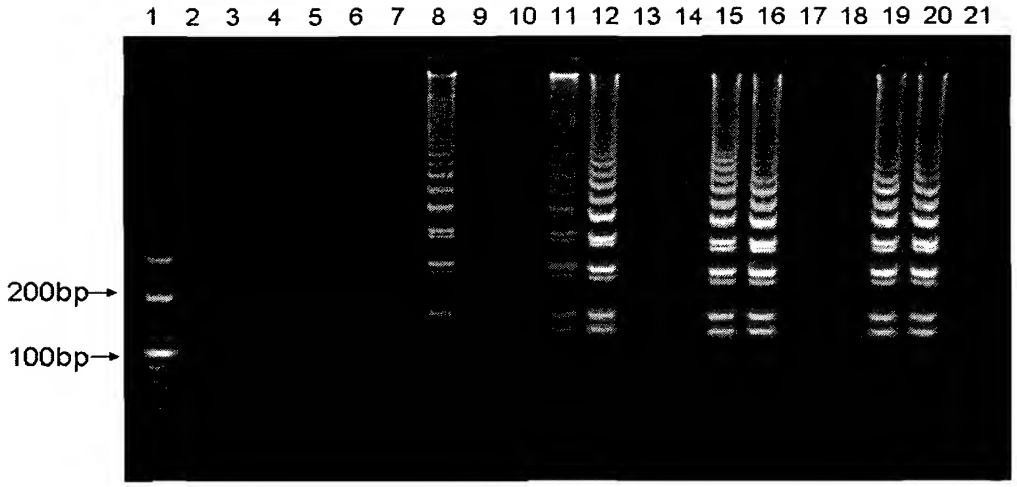
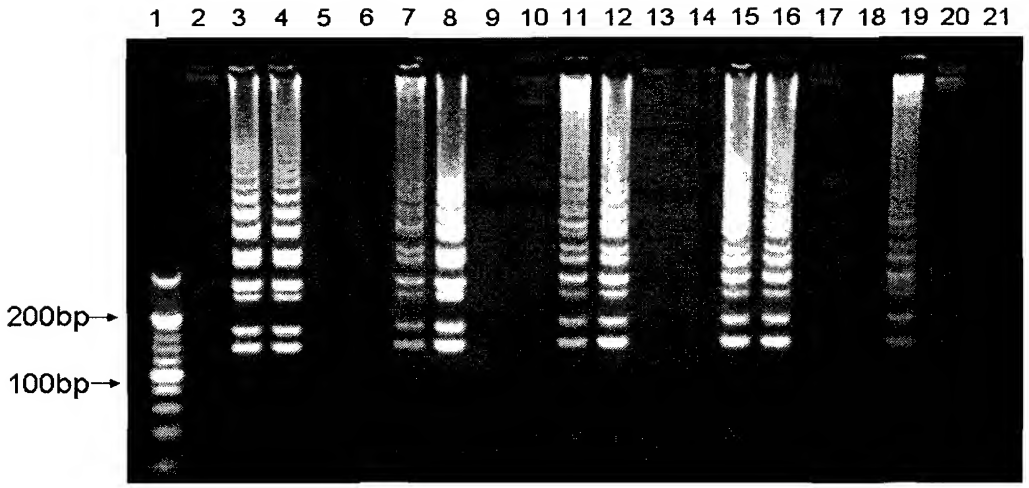

11 agcatcctcattttatgtccaacatcagagacttaatactgaacaaatgccacataaaggtaatgactgt
3'-end region common to each of sense primers SY153L
5'-end region of SY153Lp13-0 Primer
5'-end region of SY153Lp13-5 Primer
5'-end region of SY153Lp13-10 Primer
5'-end region of SY153Lp13-15 Primer
5'-end region of SY153Lp10 Primer
5'-end region of SY153Lp13 Primer
5'-end region of SY153Lp16 Primer
5'-end region of SY153Lp22 Primer
5'-end region of SY153Lp25 Primer
5'-end region of SY153Lp28 Primer
81 tgaagaagatttaacttaacatcttgcagcatcactaagaactcgctttatactcagtgcttttgggttg
5'-end region of SY153Rp13-0 Primer
3'-end region common to each of reverse primers SY153R
5'-end region of SY153Rp13-5 Primer
5'-end region of SY153Rp13-10 Primer
5'-end region of SY153Rp13-15 Primer
5'-end region of SY153Rp10 Primer
5'-end region of SY153Rp13 Primer
5'-end region of SY153Rp16 Primer
5'-end region of SY153Rp22 Primer
5'-end region of SY153Rp25 Primer
5'-end region of SY153Rp28 Primer

Figure 2



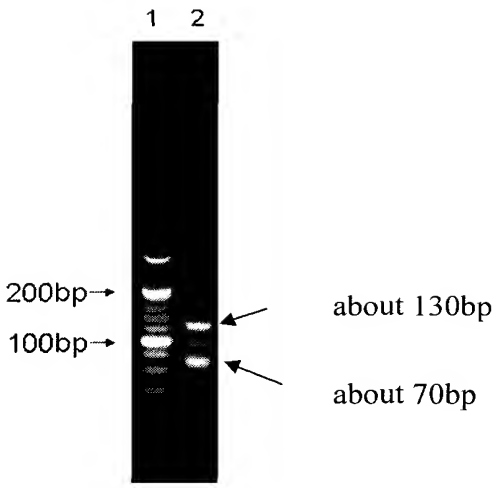
Lane	Primer Set	Template Present (Y/N)	Reaction Time (minutes)
1	DNA size marker		
2	Primer set 1	y	20
3	Primer set 1	y	40
4	Primer set 1	y	60
5	Primer set 1	n	60
6	Primer set 2	y	20
7	Primer set 2	y	40
8	Primer set 2	y	60
9	Primer set 2	n	60
10	Primer set 3	y	20
11	Primer set 3	y	40
12	Primer set 3	y	60
13	Primer set 3	n	60
14	Primer set 4	y	20
15	Primer set 4	y	40
16	Primer set 4	y	60
17	Primer set 4	n	60
18	Primer set 5	y	20
19	Primer set 5	y	40
20	Primer set 5	y	60
21	Primer set 5	n	60

Figure 3



Lane	Primer Set	Template Present (Y/N)	Reaction Time (Minutes)
1	DNA size marker		
2	Primer set 6	y	20
3	Primer set 6	y	40
4	Primer set 6	y	60
5	Primer set 6	n	60
6	Primer set 7	y	20
7	Primer set 7	y	40
8	Primer set 7	y	60
9	Primer set 7	n	60
10	Primer set 8	y	20
11	Primer set 8	y	40
12	Primer set 8	y	60
13	Primer set 8	n	60
14	Primer set 9	y	20
15	Primer set 9	y	40
16	Primer set 9	y	60
17	Primer set 9	n	60
18	Primer set 10	y	20
19	Primer set 10	y	40
20	Primer set 10	y	60
21	Primer set 10	n	60

Figure 4



Legend for Figure 4
Lane 1: DNA size marker
Lane 2: amplified products of sY153 treated with a restriction enzyme

I, Takefumi Ishidao, declare under the penalty of perjury of the laws of the United States of America that the foregoing is true and correct to the best of my information and belief.

Signed this 15th of February, 2010, at Yokohama JAPAN

Takefumi Ishidao

Takefumi ISHIDAO